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(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: WELLS, Andrew, Stephen [GB/GB]; SmithKline Beecham Pharmaceuticals, Old Powder Mills, Leigh, Tonbridge, Kent TN11 9AN (GB).

(74) Agent: GIDDINGS, Peter, John; SmithKline Beecham plc, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

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(54) Title: ENZYMATIC RESOLUTION OF BENZODIAZEPINE–ACETIC ACID ESTERS WITH A LIPASE

(57) Abstract

The present invention involves enantiomerically pure benzodiazepine acetic acid esters and methods of preparing them by resolving a racemic mixture utilizing a lipase.

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TITLE OF INVENTION

ENZYMATIC RESOLUTION OF BENZODIAZEPINE-ACETIC ACID ESTERS WITH A LIPASE

FIELD OF THE INVENTION

This invention relates to the use of a lipase enzyme to effect the resolution of optical isomers of a chemical compound.

BACKGROUND OF THE INVENTION

The use of proteolytic enzymes to hydrolyze esters is well known. In some cases, when the substrate ester possesses one or more chiral carbon atoms, the proteolytic enzyme reacts much more rapidly with one enantiomer of the racemic mixture than the other enantiomer. With appropriate substrates this chemical selectivity has been used as the basis for resolution of such a mixture. The products of such a selective hydrolytic reaction are a carboxylic acid and an alcohol for the reactive enantiomer, while the unreactive enantiomer persists as the ester. The ease of separation of the ester and acid then becomes the basis for the stereochemical purification of the carboxylic acid or alcohol fragment.

Generally, where the chirality and complexity resides in the carboxylic acid fragment of the molecule, esterases or proteases are used to prepare the corresponding chiral acid, and where the complexity and chirality reside in the alcohol fragment, lipases are used to prepare chiral alcohols. Nevertheless, some examples of lipases resolving chiral acids are known.

The stereochemical purity of the process is generally dependent upon the relative rates of hydrolysis of the each of the isomers of the racemate, e.g., the greater the difference in relative rates, the higher the purity of the final chiral product. The choice of

an appropriate enzyme to selectively hydrolyze a given chemical compound is largely empirical, if an appropriate enzyme can be found at all. Thus, in order to be useful, the enzyme must accept the desired compound as a substrate, selectively hydrolyze only the appropriate enantiomer, and produce an acceptable enantiomeric excess (e.e.).

Certain benzodiazepine compounds which have pharmaceutical utility are disclosed in WO 93/00095 (PCT/US/92/05463) and WO 94/14776 (PCT/US93/12436), WO 95/18619 (PCT/US95/00248) and PCT/US96/11108. As is often the case with such compounds, the pharmacological activity resides principally in one enantiomer of the racemates reported therein. Thus, it is desirable to have methods for preparing the non-racemic compounds. The processes reported therein rely upon separation of enantiomers by chiral high performance liquid chromatography (HPLC), and homochiral syntheses. However, HPLC methods generally suffer the disadvantage that they are difficult to carry out on a large scale, while chiral syntheses rely upon expensive chiral synthons and may suffer from parital racemisation during the synthetic sequence. Accordingly, new stereoselective methods for preparing such compounds are needed.

It has now been discovered that certain of the compounds reported in WO 93/00095 (PCT/US/92/05463), WO 94/14776 (PCT/US93/12436), and WO 95/18619 (PCT/US95/00248) may be prepared in nonracemic form by a process which involves resolution of a racemic mixture by stereoselective hydrolysis using a lipase isolated from the yeast *Candida Antarctica*. This resolution is facile and highly selective, and represents a considerable breakthrough in the field of chiral 3-oxo-1,4-benzodiazepine acetic acid chemistry. Resolution of racemic benzodiazepines has not previously been reported using enzymatic hydrolysis.

SUMMARY OF THE INVENTION

In one aspect, this invention is a process for resolving certain racemic substituted 3-oxo-2,3,4,5-1H-tetrahydro-1,4-benzodiazepine-2-acetic acid esters using a lipase from *Candida Antarctica* to selectively hydrolyze one of the chiral esters

In another aspect this invention is a substantially enantiomerically pure substituted 3-oxo-2,3,4,5-1H-tetrahydro-1,4-benzodiazepine-2-acetic acid compound, particularly one prepared from a racemic compound by an enzymatic hydrolysis process.

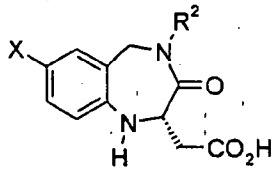
In yet another aspect, this invention is a method for improving the stereochemical purity of an ester of a 3-oxo-2,3,4,5-1H-tetrahydro-1,4-benzodiazepine-2-acetic acid.

In still another aspect, this invention comprises specific intermediate compounds which are useful in the synthesis of pharmaceutical products.

Finally, this invention is stabilised immobilised *Candida Antarctica* lipase B preparation, and a process for producing such a preparation.

DETAILED DESCRIPTION

This invention is a process for preparing a compound of the formula (I):



(I)

wherein

X is H, halogen, CO_2R^3, OR^4, COR^5, or a fibrinogen or vitronectin antagonist side chain;

R^2 is C_{1-6}alkyl, optionally substituted by Ar, Het or C_{1-6}cycloalkyl;

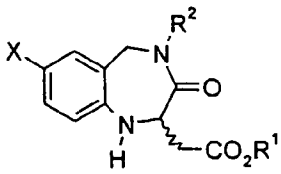
R^3 is C_{1-6}alkyl or benzyl;

R^4 is C_{1-6}alkyl, COR^3 or benzyl;

R^5 is 4,4'-bipiperidin-1-yl, (1'-benzyloxycarbonyl)-4,4'-bipiiperidin-1-yl, or (1'-t-butoxycarbonyl)-4,4'-bipiperidin-1-yl;

which comprises:

treating a compound of the formula (II):



(II)

wherein

R^1 is C_{1-20}alkyl or C_{3-20}alkenyl, optionally substitutedby Ar, NR_2 or NR_3^+, wherein R is C_{1-4}alkyl;

with *Candida Antarctica* lipase B; and

separating the resulting (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid carboxylic acid from the corresponding (R)-ester.

The 3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine acetates of formula (I) are of interest as pharmaceutical products or intermediates in the preparation of pharmaceutical products. As reported in WO 95/18619 (PCT/US95/00248), the (S) stereoisomer of these compounds is responsible for the pharmacological activity. Thus, either a homochiral synthesis, a physical separation, or chemical resolution is required. While physical resolutions and homochiral syntheses have been reported for such compounds, no chemical resolution has been achieved.

The *Candida Antarctica* lipase B has now been found to react with optionally 7-substituted 3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine acetates giving the (S)- acid with high stereoselectivity. The (R)-enantiomers are effectively not hydrolytic substrates for the enzyme hence the hydrolysis stops spontaneously at about 50% conversion. The enzyme is also highly sensitive toward the specific substitution of the (S)-substrate. For instance, the substitution of a methylene group for the 1-nitrogen of the 1,4 benzodiazepine abolishes activity. Likewise, when the X substituent is CO_2R^3, moving the X substituent from the 7-position to the 8-position results in a loss of hydrolytic activity. Nevertheless, considerable diversity appears to be tolerated at position 7 of the benzodiazepine ring. Suitably, in formula (II), R^1 is C_{1-12}alkyl or C_{1-12}alkenyl, optionally substituted by phenyl. More suitably, R^1 is C_{1-4}alkyl or benzyl. Suitably, R^2 is H, or C_{1-4}alkyl, optionally substituted by phenyl. Preferably, in formula (II), R^1 is methyl and R^2 is methyl. Suitably, X is hydrogen, bromo, iodo, t-butoxycarbonyl, benzyloxycarbonyl, methoxycarbonyl, hydroxy, methoxy, (4,4'-bipiperidin-1-yl)carbonyl, or [1'-t-butoxycarbonyl-(4,4'-bipiperidin-1-yl)]carbonyl. R^3 is preferably t-butyl.

The enzyme may be used as a part of a whole cell culture, an enzyme extract, an isolated enzyme, or an isolated enzyme attached to a solid support, such as a macro-

porous acrylic resin. Supported preparations of the *Candida Antarctica* lipase B may be purchased commercially from Novo Nordisk, Badsvaerd, Denmark (as Novozym 435) and from Boehringer Mannheim Gmbh, Mannheim, Germany (as L-2 lipase). The enzyme is generally thermostable and tolerant of high concentrations of many organic solvents. Supported preparations of the enzyme are preferred for their ease of handling.

However, certain problems may occur when a supported preparation of the enzyme is used, particularly in cases where the enzyme is non-covalently bound to resin. For instance, it has been found that due to protein leaching, the number of times a resin-supported enzyme may be reused may be somewhat limited. In addition, it has been found that when reactions are run on a large scale, emulsions may be formed by protein leaching.

It has been discovered that these problems may be overcome by pretreatment of the resin with a cross-linking reagent, such as glutaraldehyde. Other cross-linking reagents, such as dimethyl suberimidate and glutaraldehyde oligomers may also be useful. Typically such pretreatment consists merely of treating the resin with glutaraldehyde in a mixture of an organic solvent, such as t-butanol, and water for several hours. Such treatment stabilises the resin, so that much less enzyme is lost from the resin with each use, so that the number of times the resin may be reused is increased. Additionally, problems with the formation of emulsions on large scale is much reduced.

The hydrolysis is generally run in water - organic solvent mixtures. A variety of organic solvents may be useful, such as acetone, methylethyl ketone, methyl isobutyl ketone, t-butanol, benzene and toluene, and the use of either a solution or two-phase system is suitable. Nevertheless, some experimentation may be necessary in choosing an appropriate solvent to match the solubility characteristics of the substrate. For instance, when one chooses an acetone-water mixture, the substrate (R,S)-methyl 7-[(1'-t-butoxycarbonyl-(4,4'-bipiperidin-1-yl))carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate is poorly reactive. But if one uses a t-butanol/water mixture, the reaction proceeds smoothly. Ketones and secondary and tertiary alcohols are particularly useful organic solvents for one phase systems. An increase in reaction rate may sometimes be achieved by the use of a two phase system, such as water and an

aromatic hydrocarbon solvent mixture, such as water and benzene, toluene, xylene or mesitylene. Water/toluene is particularly suitable.

The reaction mixture can be buffered or run with a constant pH by the addition of base. There is generally little change in reaction rate or stereoselectivity between pH 6.0 and 8.0, however.

Suitably, the reaction is run above 20°C. Generally, if the reaction is run above room temperature, such as about 28 - 45°C the amount of solvent and the reaction time may be reduced. For instance, if the reaction is run at about 36°, 200 volumes of solvent may be reduced to about 40 volumes of solvent, and the reaction time also drops to ~24 h from 4 days.

Typically the reaction is run until 50% of the starting ester is consumed. This may be monitored by HPLC, preferably chiral HPLC. Since the (R)-ester is a very poor substrate for the enzyme, the reaction time is not critical. Typically the reaction is run from several hours to several days depending upon the temperature, solvent and substrate used in the reaction. Ten to twenty-four hours is usually suitable.

Although a large number of other lipases have been examined as potential enzymes for the their selective action upon the 3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine compounds of this invention, only the *Candida Antarctica* lipase B accepted this ring system as a selective hydrolytic substrate. For instance, the reaction failed when lipases from the following sources were tested: Porcine Pancreatic lipase, Candida lipolytica, Candida Cylindracea, Mucor Javanicus, Pseudomonas Fluorescens, Aspergillus usamii, Geotrichum Candidum, Aspergillus Niger, Humicola Lanuginosa, Ammano SAM2, Rhizopus Arrhizus, Penicillium cycopum, Rhizopus Nivens, Rhizopus Javanunicus, Ammano Lipase A, Rhizopus Delewar, Penicillinium Roqueforti, and Boehringer Lipases- L-1, L-3, L-4, L-5, L-6, and L-8. A second lipase from *Candida Antarctica*, lipase A, was also unreactive.

Other hydrolytic enzymes, such as Carlsberg subtilisin and pig liver esterase, will hydrolyse the methyl ester of the 7-unsubstituted benzodiazepine but show little stereoselectivity.

Conventional techniques for separating an ester from an acid are used to separate the carboxylic acid product from the unreacted ester. Typically, the reaction mixture is

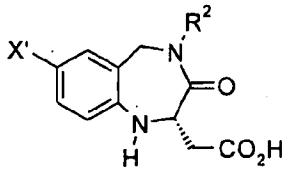
adjusted to a basic pH (e.g., > pH7) and high salt concentration, and extracted with an appropriate organic solvent. This results in the unreacted ester being dissolved in the organic solvent and the carboxylic acid product as being present in the aqueous layer. Drying and evaporation of the organic solvent yields the chiral ester; while acidification of the aqueous layer, extraction, drying and evaporation of the organic solvent yield the desired chiral acid. Other techniques such as the use of crystallisation, or chromatography over a silica gel based support, or an ion-exchange or other appropriate resin may be also be used to separate the acid from the ester, and are within the scope of this invention.

Other related techniques may be used for separating specific ester substrates from the resulting carboxylic acid product. For instance, methyl (R)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid is highly soluble in water, so that it is difficult to extract from the aqueous layer even at basic pH. In such a case the mixture of the acid and ester products of the reaction may be treated with a reactive reagent which will react preferentially with one substrate to facilitate the separation. For instance, it has been discovered that treatment of the mixture of (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid and its corresponding (R) methyl ester isomer at around pH 7.0 with carbobenzyloxy-chloride, t-butyloxycarbonyl-chloride, or the corresponding anhydrides, results in preferential acylation of the piperidinyl ring of the ester. Once the piperidine ring has been acylated, the ester is then preferentially soluble in an organic solvent and it can be separated by extraction.

Suitably the product prepared by the process of this invention will be substantially enantiomerically pure. Typically it will be greater than 80% (e.e.), preferably greater than 90%, more preferably greater than 95%, and most preferably greater than 99%.

While this invention is principally a process for preparing a non-racemic 3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine product according to formula (I), it should also be appreciated that the instant invention may also be used as a process for increasing the stereochemical purity of a chiral compound according to formula (I) wherein a significant amount of the (R)-enantiomer is present. For instance,when a chemical synthesis or a physical method, such as chromatography, has yielded a product of unsatisfactory enantiomeric purity, the instant process may be utilised to increase the e.e. of the product.

In a particular embodiment, this invention is a process for preparing a compound according to formula (III):



(III)

wherein

X' is H, halogen, CO_2R^3, or OR^4;

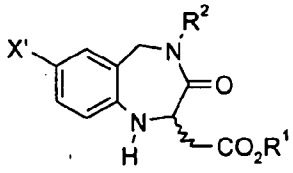
R^2 is C_{1-6}alkyl, optionally substituted by Ar, Het or C_{1-6}cycloalkyl.

R^3 is C_{1-6}alkyl or benzyl;

R^4 is C_{1-6}alkyl, COR^3 or benzyl;

which comprises:

(a) treating a compound of the formula (IV):



(IV)

wherein

R^1 is C_{1-20}alkyl or C_{3-20}alkenyl, optionally substitutedby Ar, NR_2 or NR_3^+, wherein R is C_{1-4}alkyl; and

R2 and X' are as defined for formula (III);

with *Candida Antarctica* lipase B, and

(b) separating the resulting (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid carboxylic acid from the corresponding (R)-ester.

In one preferred embodiment, this invention is a process for preparing (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, which comprises treating (R,S)-methyl 7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate with a lipase B from *Candida Antarctica*; and separating the (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid

from the (R) methyl 7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate. Other variations of this preferred embodiment, include those wherein the substrate is (R,S)-methyl 7-[(1'-t-butoxycarbonyl-(4,4'-bipiperidin-1-yl))carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate, or (R,S) methyl 7-[(1'-t-benzyloxycarbonyl-(4,4'-bipiperidin-1-yl))carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate, further comprising the step of removing the t-butoxycarbonyl or benzyloxycarbonyl group following separation of the enantiomers. Such protecting groups are generally removed by conventional means, such as acid treatment or catalytic hydrogenation.

In another preferred embodiment, this invention is a process for preparing (S) 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, which comprises treating (R,S) methyl 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate with lipase B from *Candida Antartica*; and

separating the (S) 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid from the (R) methyl 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate.

In yet another preferred embodiment, this invention is a process for preparing (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, which comprises treating (R,S)-methyl 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate with lipase B from *Candida Antartica*; and

separating the (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid from the (R)-methyl 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate.

In yet another aspect, this invention is a process for preparing a nonracemic compound according to formula (I), wherein X is $-COR^5$, or a fibrinogen or vitronectin antagonist side chain, which comprises converting a compound of formula (I), wherein X is H, halogen, CO_2R^3 or OR^4.

Compounds of formula (III) are particularly useful intermediates in preparing compounds according to formula (I) wherein X is COR^5, or a fibrinogen or vitronectin

antagonist side chain. Preferred moieties for X' are H, Br, I, CO_2CH_3, CO_2-*t*-Bu and OH. Preferred moities for R^1 are C_{1-4}alkyl and benzyl.

Specific useful compounds prepared by the processes disclosed herein are: (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, (S)-7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid; (S)-7-bromo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid; or (S)-7-t-butoxycarbonyl-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, and simple esters thereof. Typical esters would be C_{1-4}alkyl, phenyl or benzyl esters, and derivatives thereof, and they may be prepared by conventional esterification reactions from the corresponding carboxylic acids.

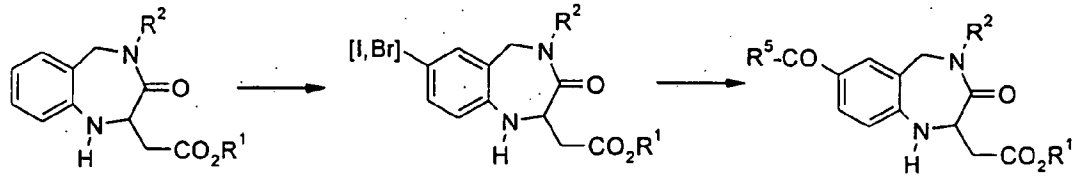
Thus, in another respect, this invention is also a method of preparing compounds of formula (I), wherein X is COR^5, or a fibrinogen or vitronectin antagonist side chain, which comprises preparing a compound of formula (III) by the above process, and converting the compound of formula (III) into a compound of formula (I), wherein X is COR^5, or a fibrinogen or vitronectin antagonist side chain. Conversion of the intermediate compounds of this invention into compounds of formula (I), wherein X is COR^5, or a fibrinogen or vitronectin antagonist side chain, may be effected by conventional reactions and procedures, such as those described in WO 93/00095 (PCT/US92/05463), WO 94/14776 (PCT/US93/12436), WO 95/18619 (PCT/US95/00248), WO 96/00730 (PCT/US95/08306), WO 96/00574 (PCT/US95/08146), PCT/US97/18001 and WO 97/24336, which are incorporated herein by reference.

In a more specific embodiment, this invention is a process for preparing a compound according to formula (I), such as (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, which comprises converting (S) 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid, (S) 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid; (S) 7-bromo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid; or (S) 7-t-butoxycarbonyl-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-

benzodiazepine-2-acetic acid, into (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid.

An exemplary procedure which may be used to convert the above intermediates into a compound of formula (I) is given by Scheme I.

Scheme I



a) pyridine•ICl or CBr_4/Ph_3P; b) CO, $Pd(OAc)_4$, Ph_3P, and (4,4'-bipiperidine or 1'-Boc-4,4'-bipiperidine or 1'-Cbz-4,4'-bipiperidine)

However, many other conventional synthetic procedures could be envisioned which would use the individual chiral intermediates of formula (III) to prepare compounds of formula (I).

Abbreviations and symbols commonly used in the chemical arts are used herein to describe the compounds which are useful in this invention. In addition, certain terms are intended to have the following meanings:

As used herein, a fibrinogen antagonist or vitronectin antagonist side chain may be generally given by the formula: $W\text{-}(CR'_2)_q\text{-}Z\text{-}(CR'R^{10})_r\text{-}U\text{-}(CR'_2)_s\text{-}V$- or $W'\text{-}(CR'_2)_q\text{-}U\text{-}(CR'_2)_s$-, wherein

R' is H, $C_{1\text{-}6}$alkyl, $C_{3\text{-}7}$cycloalkyl-$C_{0\text{-}4}$alkyl or Ar-$C_{0\text{-}4}$alkyl;

R" is R', -C(O)R' or -C(O)OR';

R^1 is H, $C_{1\text{-}6}$alkyl, $C_{3\text{-}7}$cycloalkyl-$C_{0\text{-}4}$alkyl or Ar-$C_{0\text{-}4}$alkyl;

R^5 is H, $C_{1\text{-}6}$alkyl, $C_{3\text{-}7}$cycloalkyl-$C_{0\text{-}4}$alkyl or Ar-$C_{0\text{-}4}$alkyl;

R^7 is H, halo, $-OR^{12}$, $-SR^{12}$, -CN, $-NR'R^{12}$, $-NO_2$, $-CF_3$, $CF_3S(O)_r$-, $-CO_2R'$, $-CONR'_2$, R^{14}-$C_{0\text{-}6}$alkyl-, R^{14}-$C_{1\text{-}6}$oxoalkyl-, R^{14}-$C_{2\text{-}6}$alkenyl-, R^{14}-$C_{2\text{-}6}$alkynyl-, R^{14}-$C_{0\text{-}6}$alkyloxy-, R^{14}-$C_{0\text{-}6}$alkylamino- or R^{14}-$C_{0\text{-}6}$alkyl-$S(O)_r$-;

R^8 is R', C(O)R', CN, NO_2, SO_2R' or $C(O)OR^5$;

R^9 is R', $-CF_3$, -SR', or -OR';

R^{10} is H, C_{1-4}alkyl or -NR'R";

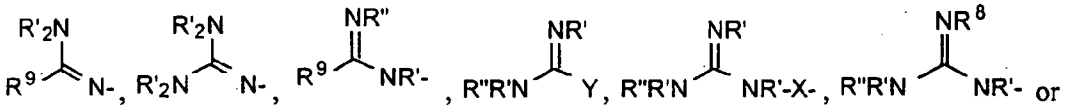
R^{12} is R', -C(O)R', $-C(O)NR'_2$, $-C(O)OR^5$, $-S(O)_mR'$ or $S(O)_2NR'_2$;

R^{14} is H, C_{3-6}cycloalkyl, Het or Ar;

R^{15} is H, C_{1-10}alkyl, C_{3-7}cycloalkyl-C_{0-8}alkyl or Ar-C_{0-8}alkyl;

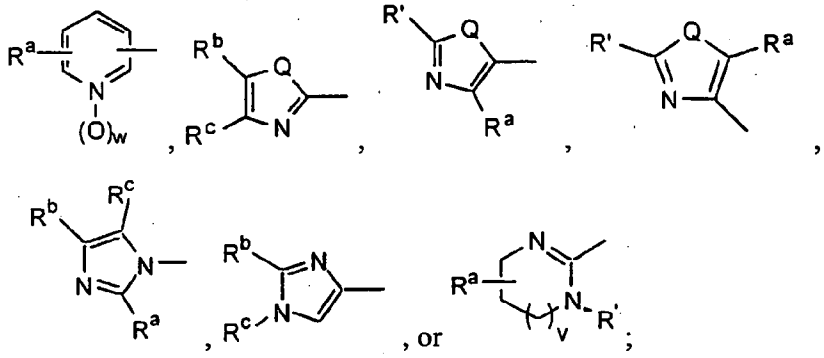
U and V are absent or CO, CR'_2, $C(=CR^{15}_2)$, $S(O)_n$, O, NR^{15}, $CR^{15}OR^{15}$, $CR'(OR")CR'_2$, $CR'_2CR'(OR")$, $C(O)CR'_2$, $CR^{15}_2C(O)$, $CONR^{15}$, $NR^{15}CO$, OC(O), C(O)O, C(S)O, OC(S), $C(S)NR^{15}$, $NR^{15}C(S)$, SO_2NR^{15}, $NR^{15}SO_2$, N=N, $NR^{15}NR^{15}$, $NR^{15}CR^{15}_2$, $NR^{15}CR^{15}_2$, CR^{15}_2O, OCR^{15}_2, C≡C, CR^{15}=CR^{15}, Het, or Ar, provided that U and V are not simultaneously absent;

W is R'R"N-, R'R"NR'N-, R'R"NR'NCO-, $R'_2NR'NC(=NR')$-, R'ONR'C(=NR')-,



;

W' is



;

Q is NR', O or S;

R^a is H, C_{1-6}alkyl, Ar-C_{0-6}alkyl, Het-C_{0-6}alkyl, or C_{3-6}cycloalkyl-C_{0-6}alkyl, halogen, OR^1, SR^1, COR^1, OH, NO_2, $N(R^1)_2$, $CO(NR^1)_2$, $CH_2N(R^1)_2$;

R^b and R^c are independently selected from H, C_{1-6}alkyl, Ar-C_{0-6}alkyl, Het-C_{0-6}alkyl, or C_{3-6}cycloalkyl-C_{0-6}alkyl, halogen, OR^1, SR^1, COR^1, OH, NO_2, $N(R^1)_2$, $CO(NR^1)_2$, $CH_2N(R^1)_2$, or R^b and R^c are joined together to form a five or six membered

aromatic or non-aromatic ring, optionally substituted by halogen, C_{1-4}alkyl, OR^1, SR^1, COR^1, OH, NO_2, $N(R^1)_2$, $CO(NR^1)_2$, $CH_2N(R^1)_2$, CN, or R"R'NC(=NR')-;

X is N=CR', C(O) or O;

Y is absent, S or O;

Z is $(CH_2)_t$, Het, Ar or C_{3-7}cycloalkyl;

m is 1 or 2;

n is 0, 1, 2 or 3;

q is 0, 1, 2 or 3;

r is 0, 1 or 2;

s is 0, 1 or 2;

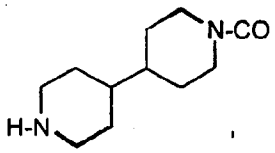
t is 0, 1 or 2;

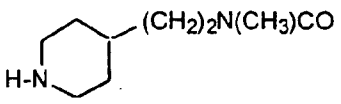
v is 0 or 1; and

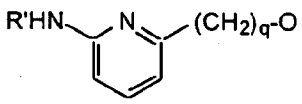
w is 0 or 1; q is 0, 1, 2 or 3.

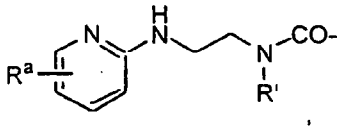
Such side chains are described, for instance, in WO 93/00095 (PCT/US92/05463), WO 94/14776 (PCT/US93/12436), WO 95/18619 (PCT/US95/00248), WO96/00730 (PCT/US95/08306), WO96/00574 (PCT/US95/08146), and PCT/US96/11108, each of which is incorporated herein by reference as though fully set forth. It will be understood that the basic nitrogen of the group W or W' may be optionally protected in conducting the process of this invention. Nitrogen protecting groups are well known to the art and comprise such groups as the acetyl, formyl, trifluoroacetyl, benzoyl, benzyloxycarbonyl and alkyloxcarbonyl groups.

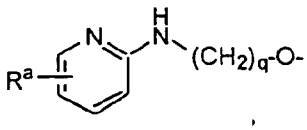
Typical fibrinogen receptor and vitronectin receptor side chains are:

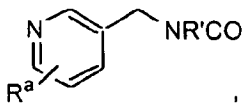


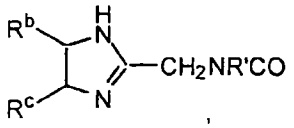


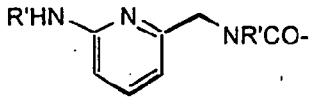


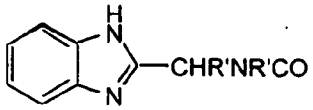


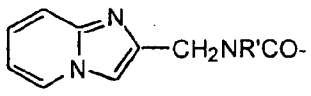


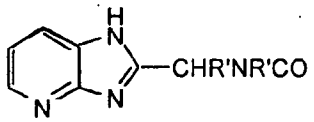




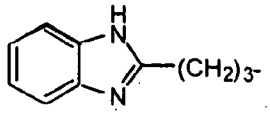








, and



C_{1-4}alkyl as applied herein is meant to include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and t-butyl. C_{1-6}alkyl additionally includes pentyl, n-pentyl, isopentyl, neopentyl and hexyl and the simple aliphatic isomers thereof. C_{1-20}alkyl likewise includes simple aliphatic hydrocarbons of the indicated number of carbons. Any alkyl group may be optionally substituted by R^7 unless otherwise indicated. C_{0-4}alkyl and C_{0-6}alkyl additionally indicates that no alkyl group need be present (*e.g.*, that a covalent bond is present).

C_{2-6}alkenyl as applied herein means an alkyl group of 2 to 6 carbons wherein a carbon-carbon single bond is replaced by a carbon-carbon double bond. C_{2-6}alkenyl includes ethylene, 1-propene, 2-propene, 1-butene, 2-butene, isobutene and the several isomeric pentenes and hexenes. C_{3-20}alkenyl likewise includes simple aliphatic hydrocarbons of the indicated number of carbons wherein one or more carbon-carbon single bonds are replaced by a carbon-carbon double bond.. Both cis and trans isomers are included. Any alkenyl group may be optionally substituted by R^7 unless otherwise indicated.

C_{2-6}alkynyl means an alkyl group of 2 to 6 carbons wherein one carbon-carbon single bond is replaced by a carbon-carbon triple bond. C_{2-6} alkynyl includes acetylene, 1-propyne, 2-propyne, 1-butyne, 2-butyne, 3-butyne and the simple isomers of pentyne and hexyne. Any sp^3 carbon atom in the C_{2-6}alkynyl group may be optionally substituted by R^7.

C_{1-4}oxoalkyl refers to an alkyl group of up to four carbons wherein a CH_2 group is replaced by a C(O), or carbonyl, group. Substituted formyl, acetyl, 1-propanal, 2-propanone, 3-propanal, 2-butanone, 3-butanone, 1- and 4-butanal groups are

representative. C_{1-6}oxoalkyl includes additionally the higher analogues and isomers of five and six carbons substituted by a carbonyl group.

A substituent on a C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl or C_{1-6}oxoalkyl group, such as R^7, may be on any carbon atom which results in a stable structure, and is available by conventional synthetic techniques.

Halogen indicates fluoride, chloride, bromide or iodide.

Ar, or aryl, as applied herein, means phenyl or naphthyl, or phenyl or naphthyl substituted by one to three moieties R^7. In particular, R^7 may be C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkthio, trifluoroalkyl, OH, F, Cl, Br or I.

Het, or heterocycle, indicates an optionally substituted five or six membered monocyclic ring, or a nine or ten-membered bicyclic ring containing one to three heteroatoms chosen from the group of nitrogen, oxygen and sulfur, which are stable and available by conventional chemical synthesis. Illustrative heterocycles are benzofuran, benzimidazole, benzopyran, benzothiophene, furan, imidazole, indole, indoline, morpholine, piperidine, piperazine, pyrrole, pyrrolidine, tetrahydropyridine, pyridine, thiazole, thiophene, quinoline, isoquinoline, and tetra- and perhydro- quinoline and isoquinoline. A six membered ring heterocycle containing one or two nitrogens, such as piperidine, piperazine, tetrahydropyridine and pyridine, are preferred heterocycles for the moiety Z. Any accessible combination of up to three substituents, such as chosen from R^7, on the Het ring that is available by chemical synthesis and is stable is within the scope of this invention.

C_{3-7}cycloalkyl refers to an optionally substituted carbocyclic system of three to seven carbon atoms, which may contain up to two unsaturated carbon-carbon bonds. Typical of C_{3-7}cycloalkyl are cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl and cycloheptyl. Any combination of up to three substituents, such as chosen from R^7, on the cycloalkyl ring that is available by conventional chemical synthesis and is stable, is within the scope of this invention.

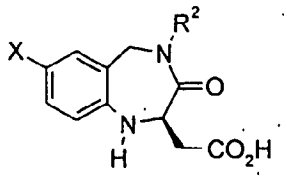
(N) as used herein indicates a nitrogen heterocycle, which may be a saturated or unsaturated stable five-, six- or seven-membered monocyclic ring, or a seven- to ten-membered bicyclic ring containing up to three nitrogen atoms or containing one nitrogen

atom and a heteroatom chosen from oxygen and sulfur, and which may be substituted on any atom that results in a stable structure. The nitrogen atom in such ring may be substituted so as to result in a quaternary nitrogen. The nitrogen heterocycle may be substituted in any stable position by R^{20}, for instance H, C_{1-4}alkoxy, F, Cl, Br, I, NO_2, NR'_2, OH, CO_2R', CONHR', CF_3, R^{14}-C_{0-4}alkyl, R^{14}-C_{1-4}alkyl-$S(O)_u$ (*e.g.*, where u is 0, 1 or 2) or C_{1-4}alkyl substituted by any of the aforementioned sustituents.. Representative of  are pyrroline, pyrrolidine, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, piperidine, piperazine, morpholine, pyridine, pyridinium, tetrahydropyridine, tetrahydro- and hexahydro-azepine, quinuclidine, quinuclidinium, quinoline, isoquinoline, and tetra- and perhydro- quinoline and isoquinoline. In particular,  may be pyridyl, pyrolidinyl, piperidinyl, piperazinyl, azetidinyl, quinuclidinyl or tetrahydropyridinyl.  is preferably 4-pyridyl, 4-(2-amino-pyridyl), 4-tetrahydropyridyl, 4-piperidinyl or 4-piperazinyl.

When R^b and R^c are joined together to form a five- or six-membered aromatic or non-aromatic ring fused to the ring to which R^b and R^c are attached, the ring formed will generally be a five- or six-membered heterocycle selected from those listed above for Het, or will be a phenyl, cyclohexyl or cyclopentyl ring. Benzimidazolyl, 4-azabenzimidazolyl, 5-azabenzimidazolyl and substituted derivatives thereof are preferred moieties for W'.

The esters of formula (II) and (IV), which are used in this invention are prepared according to procedures describe in WO 93/00095 (PCT/US92/05463), WO 94/14776 (PCT/US93/12436), WO 95/18619 (PCT/US95/00248), WO96/00730 (PCT/US95/08306), WO96/00574 (PCT/US95/08146), WO 97/24336, and PCT/US96/11108, each of which is incorporated herein by reference.

It will also be apparent to one skilled in the art that the process of the present invention may be used to prepare compounds of formula (V):



(V)

wherein R^1, R^2 and X are as described for formula (II), and that these compounds may be hydrolyzed by conventional methods to obtain the nonracemic (R) carboxylic acid isomer of formula (II) wherein R^1 is H.

The examples which follow are intended to in no way limit the scope of this invention, but are provided to illustrate how to make and use the compounds of this invention. Many other embodiments will be readily apparent and available to those skilled in the art.

Examples

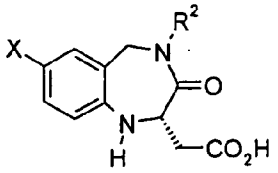
Example 1

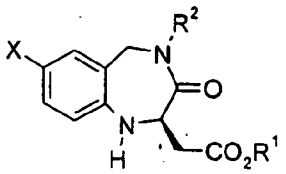
General Procedure for hydrolysis with *Candida Antartica* lipase B

The methyl 3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine-2-acetate (0.5 g), or a suitably substituted derivative thereof, was dissolved in acetone (30 mL) or t-butanol and buffer (70 mL, pH 7.0, 0.1 N phosphate). *Candida Antarctica* lipase, supported on macroporous acrylate resin (200 mg, /700 PLU/g, marketed as Novozym 435) was added and the reaction stirred at ambient for 4.0 days. The reaction was monitored by HPLC and stopped spontaneously at 47% hydrolysis. The pH was adjusted to 8.0 with NaOH solution and EtOAc added (75 mL). The mixture was filtered to remove the enzyme catalyst and the EtOAc layer separated. The aqueous layer was re-extracted with EtOAc (2 x 75 mL). The combined EtOAc extracts were dried (Na_2SO_4) and the EtOAc evaporated in vacuo to leave the (R)-ester (0.28 g). The stereochemistry was assigned by comparison to authentic (R)-ester (chiral HPLC); e.e. >99%.

The aqueous phase was adjusted to pH 5 with concentrated HCl and extracted with EtOAc (5 x 75 mL). The combined EtOAc extracts were dried (Na_2SO_4) and evaporated to leave the (S) acid (0.23 g). The e.e. was 99% (calculated from chiral HPLC).

The following 3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine-2-acetic acids were resolved using the above procedure.





X	R^1	e.e.	
H	CH_3	(S) 99%	(R) 99%
Br	CH_3	(S) 99.4%	(R) 99.4%
I	CH3	(S) 98%	(R) 95%
CO_2-t-Bu	CH_3	(S) 99.8%	(R) 99.1%
[1'-Cbz-(4,4'-bi-piperidin-1-yl)]carbonyl	CH_3	(S) 98.3%	

Example 2

Preparation of (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid

(R,S) methyl 7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate (34g, 0.077 mol) was slurried in water (580 mL) and 1.0N HCl (34 mL). The mixture was stirred at 30°C for 0.5 hr maintaining the pH at ~6.2 by the addition of 1.0N HCl. Immobilised lipase resin (Boehringer L-2 lipase, 8.2 g) was added and the reaction stirred at 30°C maintaining the pH at 6.2 by the addition of 1.5M NH_3 solution. When the demand for titrant ceased, the enzyme resin was filtered off and washed with water (50 mL).

The filtrate was treated with a solution of benzyl chloroformate (7.8 g, 0.046 mol) in CH_2Cl_2 (130 mL). This mixture was stirred at 30°C for 1 hr keeping the pH at 7.0 by adding 1.5 molar NH_3 solution. The phases were separated and the aqueous phase re-extracted with CH_2Cl_2 (130 mL).

The aqueous phase was heated at 55°C under vacuum keeping the pH at 6.8. When the volume of the solution was ~150 mL, the solution was cooled to 5°C and left for 12 hr with occasional pH adjustment. The solid was then filtered off, washed with cold water and dried to yield the title compound (16g, 80%); 99.9% (S)-isomer by chiral HPLC analysis.

The dichloromethane extracts were evaporated to yield the title compound (R)-methyl 7-[1'-benzyloxycarbonyl-(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid as a pale yellow crystalline solid (23g,100%); >98% (R)-isomer by chiral HPLC analysis.

Example 3

Preparation of methyl (S)-7-[[(N'-benzyloxycarbonyl)-4,4'-bipiperidinyl]carbonyl]-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepine-2-acetic acid

a) methyl 7-[[(N'-benzyloxycarbonyl)-4,4'-bipiperidinyl]carbonyl]-3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine-2-acetate

A mixture of methyl 7-iodo-3-oxo-2,3,4,5-tetrahydro-1,4-benzodiazepine-2-acetate (50 g, 134 mmol), 1'-benzyloxycarbonyl-4,4'-bipiperidine hydrochloride (52.12 g, 154 mmol), Hunigs base (67.9 mL, 402 mmol), NMP (200 mL), water (5.2mL, 289 mmol) and palladium II chloride bistriphenylphosphine (1.88g, 2.68 mmol) was shaken in a Parr shaker at 95°C under carbon monoxide at 8 - 14 psi. After 4 h carbon monoxide uptake ceased and the reaction mixture was allowed to cool to room temperature. Dichloromethane (400 mL) was then added and the solution was filtered and washed with water. The organic layer was concentrated to dryness and the residue was slurried in methanol (770 mL). The product was filtered and washed with methanol and sucked dry to afford the title compound (54 g, 70%).

b) (S)-7-(4,4'-bipiperidinyl)carbonyl-3-oxo-2,3,4,5-tetrahydro-1H-1,4-benzodiazepine-2-acetic acid

The compound of Example 3(a) was dissolved in t-butanol and buffer (70 mL, pH 7.0, 0.1 N phosphate). *Candida Antarctica* lipase B, supported on macroporous acrylate

resin (200 mg, /700 PLU/g, marketed as Novozym 435) was added and the reaction stirred at ambient temperature for 4.0 days. The pH was adjusted to 8.0 with NaOH solution and EtOAc added (75 mL). The mixture was filtered, and the EtOAc layer separated. The aqueous layer was re-extracted with EtOAc, the combined EtOAc extracts were dried (Na_2SO_4) and the EtOAc evaporated in vacuo to yield the (R)-ester.

The aqueous phase was adjusted to pH 5 with HCl and extracted with EtOAc. The combined EtOAc extracts were dried (Na_2SO_4) and evaporated to leave the (S)-acid (99% e.e.)

A solution of the (S) acid (0.15 mmol) in methanol (40 mL) and acetic acid (8 drops) was shaken in a hydrogen atmosphere (45 psi) with 10% Pd/C (20 mg) for 30 min. The mixture was filtered through CELITE® and the filtrate concentrated *in vacuo* to yield the title compound.

Example 4

Preparation of (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid

(R,S)-methyl 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid (20 g, 0.081 mol) was slurried in toluene (200 mL) and heated to reflux for five minutes. The solution was cooled to 40°C and water (200 mL) and Novozym 435 added (20 g). The reaction was stirred at 40°C, maintaining the pH at 7.0 with ammonia solution (1.5 mols). After sixteen hours, HPLC analysis showed complete reaction. The enzyme resin was filtered off and washed with warm water (45 mL) and warm toluene (75 mL).

The toluene layer was separated and the aqueous layer was extracted with ethyl acetate (2 x 100 mL) maintaining the pH at 8.0 with ammonia solution (1.5M). The combined organic extracts (toluene and ethyl acetate) were stripped to dryness and the residue recrystallised from dichloromethane - hexane to yield the (R)-isomer of the starting ester (8 g, 80%).

The aqueous layer is layered with ethyl acetate, acidified to pH 4.0 with hydrochloric acid, striped to dryness, and recrystallised to yield the title compound.

Example 5

Preparation of (S)-7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid

The procedure of Example 3 was repeated to the point of extracting the (R)-ester from the aqueous phase containing (S) 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid. The aqueous solution was then adjusted to pH 7.0 and pyridine ICl complex (10.2 g, 0.042 mol) was added. The mixture was stirred for one hour maintaining the pH at 6.0 with ammonia solution (1.5 M). The pH was then adjusted to 4.0 with HCl (conc) and stirred for sixteen hours. The product was filtered off and washed with cold pH 4 buffer and dried to yield the title compound (11.2 g, 84%). 99.82% (S)-isomer by chiral HPLC analysis.

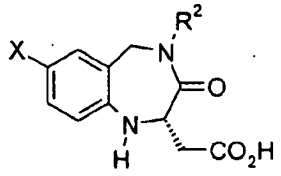
Example 6

Preparation of a stabilised supported enzyme

Novozym 435 (Novo Nordisk, 3 g) was stirred at room temperature in a mixture of water (4 mL), t-butanol (45 mL) and glutaraldehyde (1 mL, 50% w/w in water) for about 3-4 h without pH adjustment. The resin was filtered and washed with water, and was used without further drying.

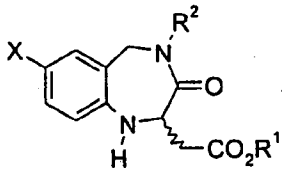
What is claimed is:

1. A process for preparing a compound of the formula (I):



(I)

X is H, halogen, CO_2R^3, OR^4, COR^5, or a fibrinogen or vitronectin antagonist side chain;
R^2 is C_{1-6}alkyl, optionally substituted by Ar, Het or C_{1-6}cycloalkyl.
R^3 is C_{1-6}alkyl or benzyl;
R^4 is C_{1-6}alkyl, COR^3 or benzyl;
R^5 is 4,4'-bipiperidin-1-yl, (1'-t-benzyloxycarbonyl)-4,4'-bipiperidin-1-yl or (1'-t-butoxycarbonyl)-4,4'-bipiperidin-1-yl;
which comprises:
(a) treating a compound of the formula (II):



(II)

R^1 is C_{1-20}alkyl or C_{3-20}alkenyl, optionally substitutedby Ar, NR_2 or NR_3^+, wherein R is C_{1-4}alkyl;

with *Candida Antartica* lipase B, and

(b) separating the resulting 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid carboxylic acid from the corresponding ester.

2. A process according to claim 1 wherein the *Candida Antartica* lipase B is fixed upon a solid support.

3. A process according to claim 2 wherein the *Candida Antarctica* lipase B enzyme is stabilised by treatment with a crosslinking agent.

4. A process according to claim 1 wherein the (S)-isomer is separated from the (R)-isomer by extraction of an aqueous solution with an immiscible organic solvent.

5. A process according to claim 1 wherein R^1 is methyl.

6. A process according to claim 1 wherein R^2 is methyl.

7. A process according to claim 1 wherein the reaction temperature is above room temperature.

8. A process for preparing (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid,
which comprises:
(a) treating (R,S) methyl 7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate with lipase B from *Candida Antartica*; and
(b) separating the (S)-7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid from the (R) methyl 7-[(4,4'-bipiperidin-1-yl)carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid.

9. A process for preparing (S) 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid,
which comprises
(a) treating (R,S) methyl 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate with lipase B from *Candida Antartica*; and
(b) separating the (S) 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid from the (R) methyl 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetate.

10. A process for preparing (S) 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid,

which comprises

(a) treating (R,S) methyl 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid with lipase B from *Candida Antartica*; and

(b) separating the (S) 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid from the (R) methyl 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid.

11. A compound which is:

(S) 2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid,

(S) 7-iodo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid;

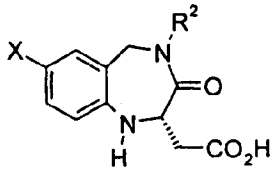
(S) 7-bromo-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid;

or

(S) 7-t-butoxycarbonyl-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid.

12. A process for preparing a compound according to formula (I), as described in claim 1 wherein X is -COR^5, or a fibrinogen or vitronectin antagonist side chain,
which comprises converting a compound according to claim 11 into said compound of formula (I).

13. A process for preparing a compound of formula (I):



(I)

wherein

X is COR^5, or a fibrinogen or vitronectin antagonist side chain;

R^2 is C_{1-6}alkyl, optionally substituted by Ar, Het or C_{1-6}cycloalkyl;

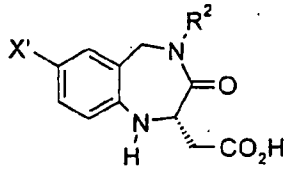
R^3 is C_{1-6}alkyl or benzyl;

R^4 is C_{1-6}alkyl, COR^3 or benzyl;

R^5 is 4,4'-bipiperidin-1-yl, (1'-benzyloxycarbonyl)-4,4'-bipiiperidin-1-yl, or (1'-t-butoxycarbonyl)-4,4'-bipiperidin-1-yl;

which comprises,

(a) preparing a compound according to formula (III):



(III)

wherein

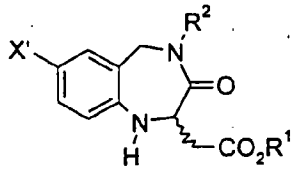
X' is H, halogen, CO_2R^3, or OR^4;

R^2 is C_{1-6}alkyl, optionally substituted by Ar, Het or C_{1-6}cycloalkyl.

R^3 is C_{1-6}alkyl or benzyl;

R^4 is C_{1-6}alkyl, COR^3 or benzyl;

by treating a compound of the formula (IV):



(IV)

wherein

R^1 is C_{1-20}alkyl or C_{3-20}alkenyl, optionally substitutedby Ar, NR_2 or NR_3^+, wherein R is C_{1-4}alkyl;

with *Candida Antarctica* lipase B, and

(b) separating the resulting (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid carboxylic acid from the corresponding (R)-ester of formula (III); and

(c) converting the compound of formula (III) into a compound of formula (I), wherein X is COR^5, or a fibrinogen or vitronectin antagonist side chain.

14. A process according to claim 14 wherein R^1 is C_{1-4}alkyl or benzyl; and X' is H, Br, I, CO_2CH_3, CO_2-*t*-Bu or OH.

INTERNATIONAL SEARCH REPORT

Ir. .ational Application No
PCT/GB 97/03522

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P41/00 C12P17/10 C12P17/16 C07D243/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 18619 A (SMITHKLINE BEECHAM CORP ;BONDINELL WILLIAM EDWARD (US); CALLAHAN J) 13 July 1995 cited in the application see claims	1
A	WO 94 14776 A (SMITHKLINE BEECHAM) 7 July 1994 cited in the application see claims	1
A	WO 93 00095 A (SMITHKLINE BEECHAM CORP) 7 January 1993 cited in the application see page 24 - page 25; claims	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
7 April 1998	16/04/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Delanghe, L

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INTERNATIONAL SEARCH REPORT

Ir. .ational Application No
PCT/GB 97/03522

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 529 929 A (ROSSI JR RICHARD F ET AL) 25 June 1996 see claims -----	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir. ational Application No
PCT/GB 97/03522

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